## Remarks

The Office Action mailed 5 August 2002 has been received and reviewed. Claims 14, 18, 25, and 28-30 having been amended, and claims 31-34 having been added, the pending claims are claims 1-34. Reconsideration and withdrawal of the rejections is respectfully requested

The amendment of claim 14 to recite "comprising an autonomous replicating sequence" is supported by the specification at, for instance, page 18, lines 27-32 .

Claim 18 has been amended to be an independent claim. The amendment of claim 18 to recite "integrative" and "comprising an autonomous replicating sequence" is supported by the specification at, for instance, page 18, lines 27-32.

The amendment of claim 25 to recite "integrated into chromosomal DNA of the yeast" is supported by the specification at, for instance, page 16, lines 14-18.

The amendments of claim 28 are supported by the specification at, for instance, page 18, lines 27-32, and by claim 28 as originally filed. The amendment of deleting the phrase "for integrating an exogenous DNA sequence including a first selection marker into chromosomal DNA of a target yeast cell" from the preamble and inserting the phrase elsewhere in the claim is made to clarify the scope of the claim. It is applicants' position that the amendment does not narrow the scope of the claim.

The amendment of claim 29 to recite "autonomous replicating sequence" is supported by the specification at, for instance, page 18, lines 27-32. The amendment of deleting the phrase "for integrating an exogenous DNA sequence into a yeast to form stable integrants which ferment xylose to ethanol" from the preamble and inserting the phrase elsewhere in the claim is made to clarify the scope of the claim. It is applicants' position that the amendment does not narrow the scope of the claim.

The amendment of claim 30 to recite "comprising an autonomous replicating sequence" is supported by the specification at, for instance, page 18, lines 27-32.

New claim 31 is supported by the specification at, for instance, page 19, line 29 through page 21, line 2.

New claims 32 and 33 are supported throughout the specification.

New claim 34 is supported by the specification at, for instance, page 22, line 1 through page 23, line 22.

The Examiner is requested to consider the following when reviewing Applicants' response to the rejections. It is well known in the art that there are several distinct types of chimeric plasmid vectors, including "(i) YIp (yeast integrating plasmids), which are unable to replicate and transform by integration into the genome of the recipient strain; (ii) YEp (yeast episomal plasmids), which carry the replication origin of the yesat 2-μm circle, an endogenous yeast plasmid, and can replicate in the recipient cell; and (iii) YRp (yeast replicating plasmids), which can replicate utilizing yeast autonomous replicating sequences (ARS)" (see Gietz et al., BioTechniques, 30, 816-831 (2001), at page 817, col. 2 [1], submitted herewith as Exhibit A).

The present application discloses at page 4, line 13-27, that exogenous gene(s) can be cloned into yeast by two separate ways. One method is to clone the exogenous gene(s) into a plasmid vector containing either a functional yeast replication origin (such as the 2 μm replicon) or an autonomous replicating sequence (ARS) that allows the plasmid to replicate autonomously in its new host, followed by transformation of the desired yeast host with the plasmid containing the cloned gene(s). The resulting yeast transformants are able to stably maintain the cloned gene in the presence of selection pressure. However, such cloned gene(s) are unstable after prolonged culture in non-selective medium.

The present application continues to disclose at page 4, line 13, through page 5, line 25, that another way to clone the exogenous gene(s) into a yeast host is to integrate the gene(s) into the yeast chromosome. The simplest way to clone a desired gene into a yeast chromosome by integration is first to clone the desired gene into a plasmid which does not contain a replication of origin or ARS (autonomous replicating sequence) but does contain a piece of the host DNA for targeting the integration to a specific site. Such integrative vectors may be linearized within

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[1] While Gietz et al. was published after the filing date of the present application, the quoted passage references a document published in 1979.

the DNA fragment homologous to the host chromosomal DNA, and increase transformation frequencies.

**35 U.S.C. §102(a) Rejection in view of Ho et al.**

The Examiner rejected claims 1-13 under 35 U.S.C. §102(a) as being anticipated by Ho et al. (WO 95/13362). This rejection is respectfully traversed. According to MPEP § 2131 a "claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference."

Independent claim 1 is drawn to a "yeast which ferments xylose to ethanol, comprising: a yeast having genes integrated at each of multiple reiterated ribosomal DNA sites of the yeast, said genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase."

Ho et al. discloses yeast transformed with plasmid constructs containing the genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase cloned into pLSK15 (a low copy number plasmid) or pUCKm10 (a high copy number plasmid) (see Ho et al., p. 15, line 29-p. 16, line 31). As explained by Ho et al., these vectors contain a yeast 2 μm replicon and replicate autonomously in *S. cerevisiae* and other yeast (see Ho et al., p.16, lines 2-3, 25, and 28-29).

In the Response to Office Action mailed August 14, 2000, Applicants argued that Ho et al. does not teach or suggest yeast having genes integrated at each of multiple reiterated ribosomal DNA sites of the yeast as recited in claims 1-13. Applicants vigorously repeat this argument. In maintaining her rejection, the Examiner simply responded with the statement that "the Ho et al. reference states that their method includes the step of introducing DNA into yeast so as to cause the yeast to have introduced genes encoding xylose reductase etc. . . . [t]hus, the reference does teach gene integration . . . ." Office Action mailed Aug. 8, 2002, p.10, (emphasis added). Applicants adamantly disagree with this statement. Ho et al. does not teach the integration of DNA into the yeast chromosome. Ho et al. merely teaches the introduction of plasmid constructs capable of autonomous replication into the yeast cytoplasm by electroporation methods (p.17, lines 8-11). The Examiner has made an unexplained and

unjustified connection between introducing a plasmid and the subsequent integration of that plasmid. Further, even if Ho et al. did teach integration, which Applicants argue it does not, Ho et al. does not teach "a yeast having genes integrated at each of multiple reiterated ribosomal DNA sites of the yeast " (claim 1). If this rejection is maintained, the Examiner is requested to provide reasoned statement as to why the Ho et al. disclosure of "introducing DNA into yeast so as to cause the yeast to have introduced genes . . . " necessarily leads the Examiner to conclude that "the reference does teach integration."

Since the disclosure of Ho et al. does not set forth each and every element of claims 1-13, Ho et al cannot anticipate claims 1-13. Withdrawal of this rejection under 35 U.S.C. §102(a) is respectfully requested.

**35 U.S.C. §102(b) Rejection in view of Le Dall et al.**

Claims 14-16, 18-19, and 28 are rejected under 35 U.S.C. §102(b) as being anticipated by Le Dall et al. (Current Genetics, vol. 26, pgs. 38-44, 1994). This rejection is respectfully traversed.

Independent claim 14 recites "transforming the cells with a replicative and integrative plasmid comprising an autonomous replicating sequence . . . ." Independent claim 18 recites "transforming yeast cells with a replicative and integrative plasmid comprising an autonomous replicating sequence . . . ." Independent claim 28 recites a "plasmid vector comprising a functional yeast autonomous replicating sequence . . . ." The plasmid vectors of Le Dall et al. contain "ori sequences for selection and replication in *E. coli*" (see p. 40, col. 1, second full paragraph). These "ori sequences" are not autonomous replicating sequences. The plasmid vectors of Le Dall et al. do not include an autonomous replicating sequence.

Claim 19 is directed to a product-by-process. "If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process" (M.P.E.P §2113). It is respectfully submitted that the product of claim 19 is not the same as a product of the prior art.

Claim 19 depends upon claim 18, which recites

> "A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising: (i) transforming yeast cells with a replicative and integrative plasmid comprising an autonomous replicating sequence . . . (ii) repeatedly replicative the transformed yeast cells from step (i) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative and integrative plasmid comprising an autonomous replicating sequence in subsequent generations of the progeny cells . . . ."

Thus, the yeast of claim 19 contains a replicative and integrative plasmid comprising an autonomous replicating sequence. Le Dall et al. does not teach a yeast that contains a replicative and integrative plasmid comprising an autonomous replicating sequence.

Since the disclosure of Le Dall et al. does not set forth each and every element of claims 14-16, 18, 19 and 28, Le Dall et al. cannot anticipate claims 14-16, 18, 19 and 28. Withdrawal of this rejection under 35 U.S.C. §102(b) is respectfully requested.

**35 U.S.C. §102(a) Rejection in view of Lopes et al.**

The Examiner rejected claims 14-16, 18, 19, 28 and 30 under 35 U.S.C. §102(a) as being anticipated by Lopes et al. (Yeast, vol. 12, no. 5, pgs. 467-477, April 1996).

Independent claim 14 recites "transforming the cells with a replicative and integrative plasmid comprising an autonomous replicating sequence . . . ." Independent claim 18 recites "transforming yeast cells with a replicative and integrative plasmid comprising an autonomous replicating sequence . . . ." Independent claim 28 recites a "plasmid vector comprising a functional yeast autonomous replicating sequence . . . ." Independent claim 30 recites "replicating cells having reiterated genomic DNA and which contain a replicative and integrative plasmid comprising an autonomous replicating sequence . . . ." The plasmid vectors of Lopes et al. based on pMIRY2 include yeast rDNA, a chloroplast DNA marker, the LEU2d gene, and pBR322 sequences, and the plasmid vectors of Lopes et al. based on pMIRY1 include yeast rDNA, a synthetic oligonucleotide, the LEU2d gene, and pUC9 sequences. The plasmid vectors of Lopes et al. do not include an autonomous replicating sequence.

Claim 19 is directed to a product-by-process, and depends upon claim 18, which recites

> "A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising: (i) transforming yeast cells with a replicative and integrative plasmid comprising an autonomous replicating sequence . . . (ii) repeatedly replicative the transformed yeast cells from step (i) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative and integrative plasmid comprising an autonomous replicating sequence in subsequent generations of the progeny cells . . . ."

Thus, the yeast of claim 19 contains a replicative and integrative plasmid comprising an autonomous replicating sequence. Lopes et al. does not teach a yeast that contains a replicative and integrative plasmid comprising an autonomous replicating sequence.

Since the disclosure of Lopes et al. does not set forth each and every element of claims 14-16, 18, 19, 28 and 30, Lopes et al. cannot anticipate claims 14-16, 18, 19, 28 and 30. Withdrawal of this rejection under 35 U.S.C. §102(a) is respectfully requested.

**35 U.S.C. §102(b) Rejection in view of Fujii et al.**

The Examiner rejected claims 14-16, 18, 19, 28 and 30 under 35 U.S.C. §102(b) as being anticipated by Fujii et al. (Applied and Environmental Microbiology, vol. 56, no. 4, pages 997-1003, April 1990).

Independent claim 14 recites "transforming the cells with a replicative and integrative plasmid comprising an autonomous replicating sequence . . . ." Independent claim 18 recites "transforming yeast cells with a replicative and integrative plasmid comprising an autonomous replicating sequence . . . ." Independent claim 28 recites a "plasmid vector comprising a functional yeast autonomous replicating sequence . . . ." Independent claim 30 recites "replicating cells having reiterated genomic DNA and which contain a replicative and integrative plasmid comprising an autonomous replicating sequence . . . ." Fujii et al. discloses that the plasmid vectors are made linear before introduction to yeast cells, and that the "fragment does not have an ARS sequence" (see paragraph at p. 998 bridging col. 1 and col. 2, and the paragraph bridging pp. 1001-1002).

Claim 19 is directed to a product-by-process, and depends upon claim 18, which recites

> "A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising: (i) transforming yeast cells with a replicative and integrative plasmid comprising an autonomous replicating sequence . . . (ii) repeatedly replicative the transformed yeast cells from step (i) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative and integrative plasmid comprising an autonomous replicating sequence in subsequent generations of the progeny cells . . . ."

Thus, the yeast of claim 19 contains a replicative and integrative plasmid comprising an autonomous replicating sequence. Fujii et al. does not teach a yeast that contains a replicative and integrative plasmid comprising an autonomous replicating sequence.

Since the disclosure of Fujii et al. does not set forth each and every element of claims 14-16, 18, 19, 28, and 30, Fujii et al. cannot anticipate claims 14-16, 18, 19, 28, and 30. Withdrawal of this rejection under 35 U.S.C. §102(b) is respectfully requested.

**The 35 U.S.C. §103(a) Rejections**

The Examiner rejected claims 1-30 under 35 U.S.C. §103(a) as being unpatentable over Yamano et al. (Journal of Biotechnology, vol. 32, pages 173-178, 1994) in view of Le Dall et al. (Current Genetics, vol, 26, pages 38-44, 1994), Fujii et al. (Applied and Environmental Microbiology, vol. 56, no. 4, pages 997-1003, April 1990) and Tantirungkij et al. (Applied Microbiology Biotechnology, vol. 41, pages 8-12, 1994). This rejection is respectfully traversed.

The burden is on the Examiner to establish a prima facie case of obviousness of the claimed invention. According to MPEP § 2143, three criteria must be met to establish a prima facie case of obviousness. First, there must be a suggestion or motivation, either in the documents themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the document or to combine document teachings. Second, there must be a reasonable expectation of success. Finally, the prior art document (or documents when combined) must

teach or suggest all the claim limitations. It is respectfully submitted that the Examiner has failed to establish a prima facie case of obviousness over the cited documents.

Yamano et al. is directed to the construction of a brewer's yeast having an α-acetolactate decarboxylase gene for the purpose of decreasing 'diacetyl-flavor' in beer. The authors teach the use of a plasmid that has been made linear for the transformation of yeast and the integration of an exogenous gene (the α-acetolactate decarboxylase gene) into the yeast chromosome. The linear fragment introduced to yeast cannot replicate in the yeast host cell and it does not include an autonomous replicating sequence. Yamano et al. teaches co-transformation of the linearized fragment along with a second plasmid, pZNEO, which is distinct from the linearized fragment carrying the exogenous α-acetolactate decarboxylase gene (see Fig. 2 of Yamano et al.). The α-acetolactate decarboxylase gene in the linearized fragment is flanked by rRNA.

Le Dall et al. is directed to the developing a multi-copy integration system for use in *Yarrowia lipolytica* (page 39, col. 1, first full paragraph). The authors teach the use of a plasmid that has been made linear for the transformation of yeast and the integration of an exogenous reporter gene (encoding alkaline extracellular protease) into the yeast chromosome. The linear fragment introduced to yeast cannot replicate in the yeast host cell as it does not include an autonomous replicating sequence. In some aspects of this document, the linear fragment used to transform yeast was flanked by rDNA.

Fujii et al., like Yamano et al., is directed to the construction of a brewer's yeast having an α-acetolactate decarboxylase gene for the purpose of decreasing 'diacetyl-flavor' in beer. The authors teach the use of a plasmid that has been made linear for the transformation of yeast and the integration of an exogenous gene (encoding α-acetolactate decarboxylase) into the yeast chromosome. The linear fragment that is used to transform yeast cannot replicate in the yeast host cell as it does not include an autonomous replicating sequence. The linear fragment used to transform yeast was flanked by rDNA.

Tantirungkij et al. is directed to the isolation of mutants on the basis of their ability to grow rapidly on xylose and the characterization of a selected strain. The authors teach yeast transformed with a plasmid, pEXGD8, having the 2μ replication origin, and carrying the xylose

reductase and xylitol dehydrogenase genes. To obtain yeast containing xylose reductase and xylitol dehydrogenase genes and having a faster growth rate, Tantirungkij et al. teach the treatment of a pEXGD transformed yeast, the parent strain H, with the chemical mutagen ethyl methane sulfonate. Of the twelve mutant strains isolated and characterized, a single strain, IM2, demonstrated the integration of the pEXGD plasmid at an unknown location on the yeast chromosome. According to the authors, the data "impl[ied] that multi-integration occurred in strain IM2" (see Tantirungkij et al. at p. 10, paragraph bridging col. 1 and col. 2). The resulting strain IM2 "showed lower specific activities of xylose reductase and xylitol dehydrogenase . . . than [the parent] strain H" (see Tantirungkij et al. at p. 9, col. 2, second full paragraph (emphasis added)). It was this strain IM2 which was selected for further analysis by the authors. Thus, Tantirungkij et al. disclose taking a strain (the parent strain H) containing the xylose reductase and xylitol dehydrogenase genes present on a plasmid, and converting the parent strain into one with (1) multi-integration of the plasmid into the yeast chromosomal DNA and (2) decreased specific activities of xylose reductase and xylitol dehydrogenase. Further, the pEXGD plasmid used by Tantirungkij et al. does not include an autonomous replicating sequence, and Tantirungkij et al. do not teach or suggest integrating a xylulokinase gene.

No motivation to combine referenced teachings.

Applicants respectfully submit that there is no motivation to combine the cited documents. It is axiomatic that motivation to combine the documents cannot be attributed to the combination itself, and the Examiner has not shown the existence in the cited documents of a motivation to combine the disclosures to produce the claimed invention.

The Action asserts that "[i]n order to obtain a higher copy number of the genes for xylose assimilation and thus higher expression levels than observed by Tantirungkij et al. it would have been obvious to modify the teachings of Fujii et al., Le Dall et al. and Yamano et al. by adding in the xylose-assimilating recombinant yeast of Tantirungkij et al." (Action, page 9). The results of modifying Fujii et al., Le Dall et al. and Yamano et al. in this way are discussed in the following paragraphs.

Both Yamano et al. and Fujii et al. disclose a brewer's yeast having an α-acetolactate decarboxylase gene. Modifying "the teachings of Fujii et al. . . . and Yamano et al. by adding in the xylose-assimilating recombinant yeast of Tantirungkij et al." (Action, page 9) would result in the yeast of Tantirungkij et al. with the α-acetolactate decarboxylase genes taught by Fujii et al. and Yamano et al. These documents do not suggest the desirability of this combination. There is no motivation to make this change.

Le Dall et al. disclose a multi-copy integration system for use in *Y. lipolytica* and insert a gene encoding alkaline extracellular protease into a *Y. lipolytica* chromosome. It is unclear if the multi-copy integration system for use in *Y. lipolytica* described by Le Dall et al. will function in the *Saccharomyces cerevisiae* used by Tantirungkij et al. Even if it did, modifying "the teachings of . . . Le Dall et al. . . . by adding in the xylose-assimilating recombinant yeast of Tantirungkij et al." (Action, page 9) would result in the yeast of Tantirungkij et al. with a gene encoding alkaline extracellular protease. These documents do not suggest the desirability of this combination. There is no motivation to make this change.

The Action also asserts that "[a]lthough Tantirungkij et al. does not teach integration into ribosomal genes it would have been obvious for one of ordinary skill in the art at the time the invention was made to place the xylose assimilation genes into a ribosomal integration vector, as taught by Yamano et al., Le Dall et al. and Fujii et al. with a reasonable expectation of success" (Action, page 9). Implicit in this assertion is the notion that a skilled person would be motivated to increase the number of xylose reductase and xylitol dehydrogenase genes in the yeast of Tantirungkij et al. and consequently increase the activity of xylose reductase and xylitol dehydrogenase. Tantirungkij et al. already teach that "multi-integration" of the plasmid encoding the xylose reductase and xylitol dehydrogenase genes may be occurring. Thus, using the "ribosomal integration vectors" of Yamano et al., Le Dall et al. and Fujii et al. to target the xylose reductase and xylitol dehydrogenase genes to ribosomal DNA would add nothing to the method of Tantirungkij et al. because both methods result in multiple insertions. Consequently, a skilled person would not be "motivated to place the xylose assimilation genes into a ribosomal integration vector, as taught by Yamano et al., Le Dall et al. and Fujii et al." (Action, page 9).

Further, Applicants respectfully submit that since the method of Tantirungkij et al. results in a strain having decreased specific activities of xylose reductase and xylitol dehydrogenase, Tantirungkij et al. teaches away from increasing the expression of xylose reductase and xylitol dehydrogenase.

Combined teachings do not teach each and every limitation of the claimed invention

Applicants submit that even if the cited documents were combined, there would be no reasonable expectation of success. Moreover, to establish a prima facie case of obviousness, the combined teachings must teach or suggest each and every limitation of the claimed invention (MPEP § 2143). It is respectfully submitted that the combined teachings of Yamano et al., Le Dall et al., Fujii et al., and Tantirungkij et al. do not teach or suggest each and every limitation of the claimed invention.

*Method claims (independent claims 14 and 30).* The method claims recite, inter alia, "transforming the cells with a replicative and integrative plasmid comprising an autonomous replicating sequence" (claim 14) and "replicating cells having reiterated genomic DNA and which contain a replicative and integrative plasmid comprising an autonomous replicating sequence" (claim 30). None of the cited documents teach or suggest the methods of claims 14 or 30 that include the use of a replicative and integrative plasmid comprising an autonomous replicating sequence. Thus, the cited documents do not teach or suggest each and every element of claims 14 and 30.

*Product (yeast) claims (independent claims 1, 23, and 25), and product-by-process claims (independent claim 19).* The claims directed to a yeast recite, inter alia, "a yeast having genes integrated at each of multiple reiterated ribosomal DNA sites of the yeast, said genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase" (claim 1), and "a yeast having multiple copies of exogenous DNA integrated into chromosomal DNA of the yeast, the exogenous DNA including genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase" (claims 23 and 25). Tantirungkij et al. teach yeast including an integrated plasmid with both the xylose reductase and xylitol dehydrogenase genes. This document does not teach

or suggest the integration of a gene encoding xylulokinase, and this deficiency is not supplemented by the cited documents. Accordingly, the cited documents do not teach or suggest each and every element of claims 1, 23, and 25.

Claim 19 is a product-by-process claim, and depends upon claim 18, which recites

> "A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising: (i) transforming yeast cells with a replicative and integrative plasmid comprising an autonomous replicating sequence . . . (ii) repeatedly replicative the transformed yeast cells from step (i) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative and integrative plasmid comprising an autonomous replicating sequence in subsequent generations of the progeny cells . . . ."

Thus, the yeast of claim 19 contains a plasmid comprising an autonomous replicating sequence. The cited documents do not teach or suggest a yeast that contains a replicative and integrative plasmid comprising an autonomous replicating sequence. Thus, the cited documents do not teach or suggest each and every element of claim 19.

*Product (vector) claims (independent claims 28 and 29).* None of the cited documents teach or suggest a "plasmid vector comprising a functional yeast autonomous replicating sequence . . . the plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of a target yeast cell" (claim 28) or a "plasmid vector comprising a functional yeast autonomous replicating sequence . . . the plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of a yeast" (claim 29). None of the cited documents teach or suggest a plasmid vector containing a functional yeast autonomous replicating sequence for use in integrating an exogenous DNA sequence into chromosomal DNA of a target yeast cell. Thus, the cited documents do not teach or suggest each and every element of claims 28 and 29.

For the reasons set forth above, Applicants submit that claims 1-30 are nonobvious over the cited documents. Accordingly, the Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-30 in view of the cited documents.

The Examiner rejected claims 1-30 under 35 U.S.C. §103(a) as being unpatentable over Ho et al. (WO/95/13362, May 18, 1995) in view of Le Dall et al. (Current Genetics, vol. 26, pages 38-44, 1994), and Fujii et al. (Applied and Environmental Microbiology, vol. 56, no. 4, pages 997-1003, April 1990). The cited documents are discussed above. This rejection is respectfully traversed.

It is Applicants' position that the Office has failed to present a prima facie case of obviousness. Specifically, the Office has not satisfied its burden in showing that the required motivation exists, and that the combined teachings must teach or suggest each and every limitation of the claimed invention.

No motivation to combine referenced teachings.

"The initial burden is on the examiner to provide some suggestion of the desirability of doing what the inventor has done. To support the conclusion that the claimed invention is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed invention or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references" (MPEP §706.02(j) (emphasis added)). Moreover, "[t]he mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination" (MPEP §2143.01 (emphasis added)).

The Action states that "neither Le Dall et al. and Fujii et al. describes yeast containing the genes for xylose fermentation multiply integrated into the ribosomal genes" and further states that "Ho et al. discloses recombinant Saccharomyces cerevisiae which contain and express the genes for xylose assimilation integrated into the genome" (Action, p. 10). From these two propositions, the Action then concludes "it would have been obvious to one of ordinary skill to place the xylose assimilation genes of Ho et al. into the ribosomal vector of Le Dall et al. and Fujii et al. with a reasonable expectation of success." The Action does not provide any suggestion of the desirability of doing what the inventor has done. The Action provides no evidence that the cited documents expressly or impliedly suggest the claimed invention, and the

Action does not provide any reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references. Further, the Action's statement that "it would have been obvious to one of ordinary skill to place the xylose assimilation genes of Ho et al. into the ribosomal vector of Le Dall et al. and Fujii et al. with a reasonable expectation of success" is conclusory, and "[b]road conclusory statements regarding the teaching of multiple references, standing alone, are not 'evidence.' " In re Dembiczak, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999).

Since there is no indication that the cited documents or the knowledge generally available to one of ordinary skill in the art provide some suggestion or motivation to modify the reference or to combine reference teachings, the Office has not met its burden of presenting a prima facie case of obviousness.

Combined teachings do not teach each and every limitation of the claimed invention

To establish a prima facie case of obviousness, the combined teachings must teach or suggest each and every limitation of the claimed invention (MPEP § 2143). It is respectfully submitted that the combined teachings of Ho et al., Le Dall et al., and Fujii et al. do not teach or suggest each and every limitation of the claimed invention.

*Method claims (independent claims 14 and 30).* The method claims recite, inter alia, "transforming the cells with a replicative and integrative plasmid comprising an autonomous replicating sequence" (claim 14) and "replicating cells having reiterated genomic DNA and which contain a replicative and integrative plasmid comprising an autonomous replicating sequence" (claim 30). None of the cited documents teach or suggest the methods of claims 14 or 30 that include the use of a replicative and integrative plasmid comprising an autonomous replicating sequence. Thus, the cited documents do not teach or suggest each and every element of claims 14 and 30.

*Product (yeast) claims (independent claims 1, 23, and 25), and product-by-process claims (independent claim 19).* The claims directed to a yeast recite, inter alia, "a yeast having genes integrated at each of multiple reiterated ribosomal DNA sites of the yeast, said genes

encoding xylose reductase, xylitol dehydrogenase, and xylulokinase" (claim 1), and "a yeast having multiple copies of exogenous DNA integrated into chromosomal DNA of the yeast, the exogenous DNA including genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase" (claims 23 and 25). None of the cited documents teach or suggest a yeast containing the genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase integrated in the yeast. Accordingly, the cited documents do not teach or suggest each and every element of claims 1, 23, and 25.

Claim 19 is a product-by-process claim, and depends upon claim 18, which recites

> "A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising: (i) transforming yeast cells with a replicative and integrative plasmid comprising an autonomous replicating sequence . . . (ii) repeatedly replicative the transformed yeast cells from step (i) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative and integrative plasmid comprising an autonomous replicating sequence in subsequent generations of the progeny cells . . . ."

Thus, the yeast of claim 19 contains a plasmid comprising an autonomous replicating sequence. The cited documents do not teach or suggest a yeast that contains a replicative and integrative plasmid comprising an autonomous replicating sequence. Thus, the cited documents do not teach or suggest each and every element of claim 19.

*Product (vector) claims (independent claims 28 and 29).* None of the cited documents teach or suggest a "plasmid vector containing a functional yeast autonomous replicating sequence . . . the plasmid vector for use in integrating an exogenous DNA sequence into chromosomal DNA of a target yeast cell" (claims 28 and 29). None of the cited documents teach or suggest a plasmid vector containing a functional yeast autonomous replicating sequence for use in integrating an exogenous DNA sequence into chromosomal DNA of a target yeast cell. Thus, the cited documents do not teach or suggest each and every element of claims 28 and 29.

For the reasons set forth above, Applicants submit that claims 1-30 are nonobvious over the cited documents. Accordingly, the Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-30 in view of the cited documents.

## Summary

It is respectfully submitted that the pending claims 1-34 are in condition for allowance and notification to that effect is respectfully requested. The Examiner is invited to contact Applicants' Representatives, at the below-listed telephone number, if it is believed that prosecution of this application may be assisted thereby.

Respectfully submitted for

Nancy W.Y. Ho et al.,

By
Mueting, Raasch & Gebhardt, P.A.
P.O. Box 581415
Minneapolis, MN 55458-1415
Phone: (612) 305-1220
Facsimile: (612) 305-1228
**Customer Number 26813**

26813

PATENT TRADEMARK OFFICE

January 6, 2003
Date

By: David L. Provence

Attorney Name: David L. Provence
Reg. No.: 43,022
Direct Dial: (612)305-1005

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By: Sue Sombroske
Name: Sue Sombroske

**APPENDIX A - SPECIFICATION/CLAIM AMENDMENTS INCLUDING NOTATIONS TO INDICATE CHANGES MADE**
**Serial No.: 09/180,340**
**Docket No.: 290.0033 0101**

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted.

## In the Claims

For convenience, all pending claims are shown below.

1. A yeast which ferments xylose to ethanol, comprising:

a yeast having genes integrated at each of multiple reiterated ribosomal DNA sites of the yeast, said genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase.

2. The yeast of claim 1 which also ferments glucose to ethanol.

3. The yeast of claim 2 which is Saccharomyces.

4. The yeast of claim 3 wherein said sites are non-transcribed DNA sites.

5. The yeast of claim 1 wherein the genes are fused to non-glucose-inhibited promoters and the yeast simultaneously ferments glucose and xylose to ethanol.

6. The yeast of claim 5 wherein the promoters do not require xylose for induction.

7. The yeast of claim 3 wherein the genes are fused to non-glucose-inhibited promoters and the yeast simultaneously ferments glucose and xylose to ethanol.

8. The yeast of claim 4 wherein the genes are fused to non-glucose-inhibited promoters and the yeast simultaneously ferments glucose and xylose to ethanol, the promoters also not requiring xylose for induction.

9. The yeast of claim 6 wherein the xylose reductase and xylitol dehydrogenase genes are from natural yeast which ferment xylose to ethanol.

10. The yeast of claim 9 wherein the natural yeast are Candida Shehatae, Pichia stipitis or Pachysolen tannophilus.

11. The yeast of claim 9 wherein the xyulokinase gene is from a yeast or bacteria.

12. The yeast of claim 11 wherein the xyulokinase gene is from Candida Shehatae, Pichia stipitis, Pachysolen tannophilus, Saccharomyces cerevisiae, Schizosaccharomyces pombe, or Escherichia coli.

13. The yeast of claim 1 having said genes integrated at least about 10 ribosomal DNA sites of the yeast.

14. (Amended) A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising:

(a) transforming the cells with a replicative and integrative plasmid comprising an autonomous replicating sequence, [having] exogenous DNA, and [including] a first selection marker; and

(b) repeatedly replicating the cells from step (a) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative and integrative plasmid in subsequent generations of the

progency cells and produce progeny cells having multiple integrated copies of the exogenous DNA.

15. The method of claim 14, wherein the plasmid DNA also includes a second selection marker for selecting cells which include the plasmid.

16. The method of claim 14 wherein the cells are yeast or eukaryotic cells, and wherein the method further includes the step of repeatedly replicating the progeny cells from step (b) to produce a number of generations of progeny cells in the absence of selection for cells which include the selection marker, so as to promote the loss of the plasmid in subsequent generations of progeny cells and recover yeast cells each containing multiple copies of the exogenous DNA integrated into its chromosomal DNA.

17. The method of claim 16 wherein the cells are yeast cells and the exogenous DNA includes genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, which also serve as the first selection marker.

18. (Amended) A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising [The method of claim 14, which comprises]:

(i) transforming yeast cells with a replicative and integrative plasmid comprising an autonomous replicating sequence, [having] exogenous DNA, and [including] a selection marker, the exogenous DNA being flanked on each end by a DNA sequence homologous to a reiterated sequence of DNA of the host;

(ii) repeatedly replicative the transformed yeast cells from step (i) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative plasmid in subsequent generations of the progeny cells and result in progeny cells each containing multiple integrated copies of the exogenous DNA; and

(iii) replicating the progeny cells from step (ii) to produce a number of generations of progeny cells in the absence of selection for cells which include the selection marker, so as to promote the loss of the plasmid in subsequent generations of progeny cells and recover yeast cells each containing multiple copies of the exogenous DNA integrated into its chromosomal DNA.

19. Yeast cells produced by the method of claim 18.

20. The yeast cells of claim 19, wherein the exogenous DNA includes genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, and the yeast cells ferment xylose to ethanol.

21. The yeast cells of claim 20, wherein said genes are fused to non-glucose-inhibited promoters which do not require xylose for induction, and wherein the yeast cells ferment glucose and xylose simultaneously to ethanol.

22. Yeast cells according to claim 21 which substantially maintain their capacity to ferment xylose to ethanol when cultured under non-selective conditions for at least 20 generations.

23. A yeast which ferments xylose to ethanol, comprising:

a yeast having multiple copies of exogenous DNA integrated into chromosomal DNA of the yeast, the exogenous DNA including genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase fused to non-glucose inhibited promoters, the yeast fermenting glucose and xylose simultaneously to ethanol and substantially retaining its capacity for fermenting xylose to ethanol for at least 20 generations when cultured under non-selective conditions.

24. The yeast of claim 23, wherein said promoters do not require xylose for induction

25. (Amended) A yeast which ferments xylose to ethanol, comprising:

a yeast having multiple copies of [an introduced] exogenous DNA [containing] integrated into chromosomal DNA of the yeast, the exogenous DNA including genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, the yeast fermenting xylose to ethanol and substantially retaining its capacity for fermenting xylose to ethanol when cultured under non-selective conditions for at least 20 generations.

26. The yeast of claim 25, wherein the promoters do not require xylose for induction

27. A method for fermenting xylose to ethanol, comprising fermenting a xylose-containing medium with a yeast of claim 1, 22, 23, 24, 25 or 26, to product ethanol.

28. (Amended) A plasmid vector [for integrating an exogenous DNA sequence including a first selection marker into chromosomal DNA of a target yeast cell, the plasmid vector containing] comprising a functional yeast [DNA replication origin] autonomous replicating sequence and [the] an exogenous DNA comprising a first selection marker, the exogenous DNA flanked on each end by a DNA flanking sequence which is homologous to a reiterated ribosomal DNA sequence of the target yeast cell, the plasmid further including a second section marker in a position other than between the DNA flanking sequences, the plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of a target yeast cell.

29. (Amended) A plasmid vector [for integrating an exogenous DNA sequence into a yeast to form stable integrants which ferment xylose to ethanol, the plasmid vector containing] comprising a functional yeast [DNA replication origin] autonomous replicating sequence and exogenous DNA including genes encoding xylose reductase, xylitol dehydrogenase, and

xylulokinase flanked on each end by a DNA flanking sequence which is homologous to a reiterated DNA sequence of the target yeast cell, the plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of a yeast to form stable integrants which ferment xylose to ethanol.

30. (Amended) A method for forming cells having multiple integrated copies of an exogenous DNA fragment, comprising:

replicating cells having reiterated genomic DNA and which contain a replicative and integrative plasmid comprising an autonomous replicating sequence and containing the exogenous DNA to produce multiple generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative and integrative plasmid in subsequent generations of the progeny cells and produce progeny cells having multiple integrated copies of the exogenous DNA.

31. (New) The yeast of claim 1 wherein the yeast maintains xylose fermenting capability after culture in non-selective medium.

32. (New) The method of claim 14 wherein the cells are yeast.

33. (New) The method of claim 30 wherein the cells are yeast.

34. (New) A plasmid vector comprising a functional yeast autonomous replicating sequence and exogenous DNA flanked on each end by a DNA flanking sequence which is homologous to a reiterated ribosomal DNA sequence of the target yeast cell, the plasmid further comprising a selection marker in a position other than between the DNA flanking sequences, the plasmid vector for use in integrating an exogenous DNA sequence into chromosomal DNA of a target yeast cell.